

04-05-04

EV325381135US

**TRANSMITTAL LETTER**  
(General - Patent Pending)Docket No.  
49865.1

In Re Application Of: Marco Eggink, et al.

Serial No.  
10/672,215Filing Date  
26 Sep 2003Examiner  
Not Yet AssignedGroup Art Unit  
1761

Title:

Xymenynic Acid Compositions, Methods for Their Production and Uses Thereof

TO THE COMMISSIONER FOR PATENTS:

Transmitted herewith is:

Certified Copy of Priority Application EP 02 028 595.3 filed 20 December 2002 (priority previously claimed).

in the above identified application.

- ☒ No additional fee is required.
- ☐ A check in the amount of \_\_\_\_\_ is attached.
- ☒ The Director is hereby authorized to charge and credit Deposit Account No. **50-2194** as described below.
- ☐ Charge the amount of \_\_\_\_\_
- ☒ Credit any overpayment.
- ☒ Charge any additional fee required.

  
Signature

Dated: 2 April 2004

Mitchell D. Hirsch, Reg. No. 54,170  
Buchanan Ingersoll PC  
1835 Market Street, 14th Floor  
Philadelphia, Pennsylvania 19103-2985

Telephone: (215) 665-3809  
CUSTOMER NUMBER: 041068

I certify that this document and fee is being deposited on \_\_\_\_\_ with the U.S. Postal Service as first class mail under 37 C.F.R. 1.8 and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Signature of Person Mailing Correspondence

Typed or Printed Name of Person Mailing Correspondence

CC:



**THIS PAGE BLANK (USPTO)**

<b>CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)</b> Applicant(s): Marco Eggink, et al.			Docket No. 49865.1	
Serial No. 10/672,215	Filing Date 26 Sep 2003	APR 02 2004 PATENT & TRADEMARK OFFICE	Examiner Not Yet Assigned	Group Art Unit 1761
Invention: Xymenynic Acid Compositions, Methods for Their Production and Uses Thereof				
I hereby certify that the following correspondence: <div>Certified Copy of Priority Application EP 02 028 595.3 filed 20 December 2002 Return Receipt Postcard</div> <p>(Identify type of correspondence)</p> <p>is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on</p> <p><u>2 April 2004</u> (Date)</p> <p><u>Jane D. Roberts</u> (Typed or Printed Name of Person Mailing Correspondence)</p> <p><u>Jane D. Roberts</u> (Signature of Person Mailing Correspondence)</p> <p><u>EV 325381135 US</u> ( "Express Mail" Mailing Label Number)</p>				
Note: Each paper must have its own certificate of mailing.				
CUSTOMER NUMBER: 041068				



**THIS PAGE BLANK (USPTO)**



**Europäisches  
Patentamt**

**European  
Patent Office**

**Office européen  
des brevets**

**Bescheinigung**

**Certificate**

**Attestation**

Die angehefteten Unterla-  
gen stimmen mit der  
ursprünglich eingereichten  
Fassung der auf dem näch-  
sten Blatt bezeichneten  
europäischen Patentanmel-  
dung überein.

The attached documents  
are exact copies of the  
European patent application  
described on the following  
page, as originally filed.

Les documents fixés à  
cette attestation sont  
conformes à la version  
initialement déposée de  
la demande de brevet  
européen spécifiée à la  
page suivante.

**Patentanmeldung Nr.    Patent application No.    Demande de brevet n°**

02028595.3

Der Präsident des Europäischen Patentamts;  
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets  
p.o.

**R C van Dijk**

**THIS PAGE BLANK (USPTO)**



Anmeldung Nr:  
Application no.: 02028595.3  
Demande no:

Anmeldetag:  
Date of filing: 20.12.02  
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

LODERS CROKLAAN B.V.  
Hogeweg 1  
1521 AZ Wormerveer  
PAYS-BAS

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.  
If no title is shown please refer to the description.  
Si aucun titre n'est indiqué se référer à la description.)

Ximénynic acid

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s)  
revendiquée(s)  
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/  
Classification internationale des brevets:

A23L1/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of  
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SI SK

**THIS PAGE BLANK (USPTO)**



20. Dez. 2002

5 Neue europäische Patentanmeldung »XIMENYNIC ACID«  
Anmelderin: Loders Croklaan BV  
Anwaltsakte: L002H02/P-EP

---

10

**XIMENYNIC ACID**

Seed nut oils from trees such as Ximenia Americana (also known as Mountain Plum or Manzanella or Cagalera or Manzana del diablo or Tallow wood) or Ximenia Africana or Santalum spictum or Santalum acuminatur (also known as Desert quandong or Sandalwood or Katunga or Burn-burn or Mangatais) or Santalum album are known to contain an acetylenic natural fatty acid with the name Ximenynic acid (or octadeca-trans-11-en-9-ynoic acid, 9allt-18:2). The oil is known to have a number of health effects such as :

- activity as leukotriene B4 inhibitor
- activity as phopholipase A2 inhibitor
- activities against peripheral vasculopathies
- 25 - activity against headaches
- activity against kidney or heart complaints
- activity against skin ulcers

(cf Biochem Biophys Acta 921 (1987) p.621 - 624 and EP 304603 and information available on Internet).

However the availability and applicability of the acid and derivatives thereof was limited due to the fact that the acid is only present in limited amounts in most of the seed oils of these trees. Moreover the acids so far were only isolated by chromatographic techniques which are not suitable for a commercial application. Therefore there existed a need to

5 make the acid available in a form wherein it could be applied  
in foods, preferably in a form wherein the acid is dissolved  
in a system directly applicable in a food. Moreover there was  
a need to make the acid available in a more concentrated form  
than available so far and to find new ways to concentrate the  
10 acids in a form wherein it could be applied in foods. Further  
it was desirable to investigate whether the acid and its  
derivatives had other health benefits, giving broader appli-  
cation.

15 We studied the above issues and as a result of this study we  
found novel blends containing Ximenynic acid or a derivative  
thereof in the form of an alkyl ester with 1 to 11 C-atoms, a  
glycerol ester (either as mono-di-or triglyceride or as a mix  
thereof), a wax ester wherein the acid is esterified with an  
20 alcohol with 12 or more C-atoms or as a food acceptable salt  
(in particular Na, K and Ca salts) in amounts that made them  
suitable for application in foods.

Therefore our invention concerns in the first instance blends  
25 comprising Ximenynic acid, or an alkyl or glycerol ester or a  
wax ester or a food acetable salt thereof, and a glyceride  
wherein the blends comprise

(i) 0.1 to 99.9 wt %, preferably 1 to 99 wt %, most pref-  
erably 2 to 98% of Ximenynic acid, originating from a natural  
30 source therefore, or an alkyl, or glycerol ester, or a wax  
ester or a food acetable salt thereof and

(ii) 0.1 to 99.9 wt %, preferably 1 to 99%, most preferably 2  
to 98% of other fatty acids or esters or salts or glycerides,  
particularly triglycerides, not akin to those present in the  
35 natural source of the Ximenynic acid.

5 The presence of glycerides not akin to the source for the Ximenynic acid makes that the blends can be designed in such a way that they are directly applicable in a food.

Blends that are obtained from Ximenia sources also contain  
10 Nervonic acid and in that instance our blends are characterised by the fact that they display a weight ratio of Ximenynic acid to Nervonic acid in the blend of between 0.5 and 5.0, preferably 0.75 - 4.0, most preferably 1.2 to 3.5. We found that for certain applications it is beneficial that  
15 Nervonic acid is present as well in the blend.

Concentrated forms of Ximenynic acid have been made by a process which will be discussed later. These concentrates comprise Ximenynic acid or an alkylester or a glycerol  
20 ester or a wax ester or a food acceptable salt thereof in a glyceride and contain at least 15% of Ximenynic acid or the alkylester or glycerol- or wax ester or salt derivative thereof, preferably at least 20% Ximenynic acid and at least 0.5% Nervonic acid or an alkylester or glycerol- or wax ester  
25 or a salt thereof, preferably at least 5% Nervonic acid or said derivative thereof.

The other glycerides that can be applied can be selected from a broad group of food grade glycerides, in particular vegetable  
30 glycerides more particular triglycerides. We prefer to use as the other glycerides, glycerides selected from the group consisting of palm oil; cocoa butter; coconut oil; palm kernel oil; CLA-glycerides; soy bean oil, olive oil; sunflower oil; rape seed oil; safflower oil; corn oil; cotton  
35 seed oil; cocoa butter equivalents or cocoa butter replacers; fish oil; borage oil, pine nut oil; coriander oil; fungal

5 oils, or high oleic varieties thereof, or fractions thereof,  
or hardened varieties thereof, or fractions of the hardened  
varieties or mixtures of one or more of these oils and fats.  
It is however also possible to use the free fatty acids  
corresponding with the hydrolysed fatty acid residues of  
10 these glycerides in our blends, in particular in combination  
with free Ximenynic acid. Also CLA (=Conjugated Linoleic  
Acid) as free fatty acid can be applied.

The blends suitably are so designed that they display the  
15 desired N-values at 5 and 35 °C for the specific application  
in a food. N-value meaning the solid fat content as measured  
by NMR-pulse on a mixture that is not stabilised (i.e. the  
mixture is first melted at 60 to 80 °C, cooled to 0 °C and  
kept at 0 °C for 30 min whereupon it is heated to measurement  
20 temperature kept on this temperature for 30 min and then the  
N-value is measured). Preferred blends display a solid fat  
content, measured by NMR pulse on a non stabilised blend at  
the temperature indicated of  $N_5 = 5$  to 80, preferably 10 to  
70 and  $N_{35} =$  less than 20, preferably 1 to 5.

25 The acids are preferably isolated from Ximenia or from Santa-  
lum sp. The glycerides are obtained by extraction from the  
seeds using a suitable technique, for example solvent extrac-  
tion or pressing. Esters or ximenynic acid can be produced by  
30 esterification with the required alcohol (including glycerol)  
using enzymic or chemical routes. Food grade salts can be  
obtained by neutralisation of the acids with the correspond-  
ing base.

35 It was further found that the presence of oxidation stabilis-  
ers could prolonge the storage properties of the acids and

5 the blends containing them. In some instances it was even found that the oxidation stabilisers had a synergistic effect on the health properties of the acids. Therefore we prefer to apply blends comprising an effective amount of an oxidation stabiliser preferably selected from the group consisting of  
10 natural or synthetic tocopherols, BHT, TBHQ, BHA, propylgallate; free radical scavengers, enzymes with anti oxidant properties and ascorbyl esters of fatty acids. Effective amounts will differ from compound to compound and also will be different for different health effects. However the effective  
15 amount can easily be determined by minor experimentation. In general the amount of anti oxidant will vary between 0.05 and 15 wt % (on blend), in particular between 0.1 and 5 wt %.

20 According to another aspect of our invention it concerns with food products and therefore our invention also comprises food products comprising an effective amount of a health component wherein the health component is Ximenynic acid, or an alkylester or a glycerolester or a wax ester or a food acceptable  
25 salt thereof.

Again effective amount can easily be determined by the man skilled in the art without undue experimentation. In general amounts of 0.5 to 10 wt % are very effective.

30

Food compositions that we prefer are foods selected from the group consisting of margarine; fat continuous or water continuous or bicontinuous spreads, fat reduced spreads; confectionery products such as chocolate or chocolate coatings or  
35 chocolate fillings or bakery fillings, ice creams, ice cream coatings, ice cream inclusions, dressings, mayonnaises,

5 cheese, cream alternatives, dry soups, drinks, cereal bars, sauces and snack bars.

The application of our novel blends and concentrates is not limited to foods. They also can be applied in food supplements. Therefore part of our invention are also food supplements comprising an effective amount of Ximenynic acid or a derivative thereof in an encapsulating material or in granules or in powder form. In particular supplements, wherein the acid is encapsulated in encapsulating material selected from the group consisting of gelatin, starch, modified starch, flour, modified flour, sugars, in particular sucrose, lactose, glucose and fructose are preferred.

We further found that Ximenynic acid or its derivatives have other novel health effects and these are also part of our invention. In particular we found that Ximenynic acid and its derivatives possessed properties that made them very suitable for use in the preparation of food compositions or food supplements with a health effect, wherein the Ximenynic acid or an alkylester or a glycerolester or a wax ester or a food acceptable salt thereof is used for the preparation of food compositions or food supplements with at least one of the following health effects:

- i) lowering or regulation of body weight
- 30 ii) prevention or treatment of insulin resistance or related disorders such as diabetes
- iii) delaying the onset of symptoms related to development of Alzheimers disease
- iv) improving memory function
- 35 v) lowering blood lipid levels
- vi) skin anti ageing benefits

5 vii) anti cancer effects.

Further, ximenynic acid has a beneficial effect on satiety.

10 However the acid also displays properties that makes the acid  
very suitable to be used in compound systems, particularly in  
food systems to improve / enhance, hardness, texture, aera-  
tion, spreadability, oral properties, mouthfeel, flavour  
impact, colour, viscosity and easiness of processing.

15 A last embodiment of our invention concerns a new proces for  
the preparation of an enriched Ximenynic acid concentrate.  
This method involves a process for the extraction and enrich-  
ment of an oil in Ximenynic acid involving the following  
steps:

20

(i) Ximenia americana nuts are crushed and boiled with  
an solvent

(ii) the extract is separated from the solids

(iii) the solvent is removed from the oil

25 (iv the oil so obtained is partially hydrolysed with a  
lipase, preferably with Candida rugosa lipase

(v) the partially hydrolysed product is split by physi-  
cal separation techniques into a fraction enriched in xi-  
menynic acid and in a fraction which is leaner in ximenynic  
30 acid.

5    **EXAMPLES****EXAMPLE 1****Extraction of oil from *ximenia americana***

500g *Ximenia americana* fruit kernels were thoroughly crushed  
 10 in a blender. The crushed kernels were put in a 3 liter  
 round-bottom flask and 1,5 liter acetone or hexane was added.  
 The mixture was boiled under reflux for 3 hours. Afterwards,  
 the solids were filtered off and the solvent was removed from  
 the resulting extract by evaporation under vacuum. The re-  
 15 sulting oil (180g) was opaque and did not become clear upon  
 heating. The oil was analyzed on its fatty acid composition  
 (see Table I).

20 **Table I.** Fatty acid composition of oil extracted from *Xime-*  
*nia americana* kernels with different solvents.

Fatty acid	Extracted in hexane (%)	Extracted in acetone (%)
C14:1	0.3	
C16:0	1.3	1.3
C18:0	1.1	1.2
C18:1	41.4	41.3
Ximenynic acid	9.0	9.1
C20:0	0.3	0.3
C20:1	1.8	1.8
C20:2	1.6	1.6
C22:0	0.8	0.8
C22:1	1.4	1.4
C24:0	2.0	2.0
C24:1	8.4	8.4
C26:0	1.9	1.8
C26:1	7.5	7.7
C28:0	0.7	0.7
C28:1	11.8	11.5
C30:1		2.2



5

**EXAMPLE 2****Enzymatical enrichment of ximenynic acid in ximenia americana**

20g *Ximenia americana* oil (extracted with acetone) and 7ml demineralized water were put in a 100 ml screwcapped glass bottle. 0.1g *Candida rugosa* lipase in 1ml demineralized water was added and the mixture was homogenized gently. The bottle was put in a shaker (150 rpm) at 35°C. At certain timepoints, samples of the mixture were taken. In these samples, the partially hydrolyzed oil and the FFA were extracted with isooctane and the isooctane was removed by N<sub>2</sub> flow. The percentage FFA in the samples was determined by potentiometric titration. The FFA were absorbed on immobilized tertiary amines and the resulting oil was analyzed on its fatty acid composition by FAME analysis (Table II).

20

**Table II.** Hydrolysis of *Ximenia americana* oil: Extent of hydrolysis and the percentage of Ximenynic acid in the remaining oil in time.

Time (hours)	Hydrolysis extent (%)	Ximenynic acid in oil (%)
0	0.0	8.7
2	29.0	11.0
4	35.5	12.9
6	45.3	13.6
24	61.3	16.9

25

5    **EXAMPLE 3**

Effect of the addition of *Santalum acuminatum* on the activity of acetylcholine esterase

Acetylcholine esterase activity was measured using 96 -well microplate reader based on Ellmans method (G.I Ellman, D. Courtney, V. Andres Jr., R.M. Featherstone Biochem. Pharmacol. 7 (1961) 88.).

*Santalum acuminatum* nuts were extracted with hexane to obtain a lipid fraction. 1g of this fraction was hydrolysed to free fatty acids by refluxing with 0.2g of potassium hydroxide in 1.5 mls of ethanol and 0.5 mls of water for 1 hour. The potassium salts were converted to free fatty acids by addition of hydrochloric acid and then extracted into hexane. The hexane extract of *santalum acuminatum* nuts and the saponified fraction of the hexane extract were dissolved in a mixture of ethanol/ tetrahydrofuran/ acetone 60/20/20 at concentrations of 100mg/ml. Serial dilutions were then made as required. These solutions were further diluted 1 to 10 using 50mM Tris-HCL buffer pH 8 *Santalum acuminatum* solutions (20µl), Acetylthiocholine iodide (25µl 15mM in water), 5,5'-dithiobis-(2-nitrobenzoic acid (125µl 3mM in 50mM Tris-HCL buffer pH 8 containing 0.1M NaCl and 0.02M MgCl<sub>2</sub>.6H<sub>2</sub>O), Tris-HCL buffer (50 µl 50mM pH 8 containing 0.1% bovine serum albumin) were placed in wells together with acetyl choline esterase from electric eel (type VI-s lyophilised powder) (20µl 1.5 µg/ml 50mM Tris-HCL pH 8 buffer containing 0.1% bovine serum albumin)

5 The absorbance was measured at 410nm every 13s for 8 times.  
The rate of change of OD of the test materials was compared  
to the blank solutions.

The results are shown in Figure 1.

10

#### EXAMPLE 4

Effect of the addition of ximenia oil saponified fraction on  
the activity of acetylcholine esterase

15 Acetylcholine esterase activity was measured using 96 - well  
microplate reader based on Ellmans method (G.I Ellman, D.  
Courtney, V. Andres Jr., R.M. Featherstone Biochem. Pharma-  
col. 7 (1961) 88.

Ximenia oil was hydrolysed to free fatty acids by refluxing  
20 1g with 0.2g of potassium hydroxide in 1.5 mls of ethanol and  
0.5mls of water for 1 hour. The potassium salts were con-  
verted to free fatty acids by addition of hydrochloric acid  
and then extracted into hexane.

25 The saponified fraction of ximenia oil was dissolved in a  
mixture of ethanol/ tetrahydrofuran/ acetone 60/20/20 at  
concentrations of 100mg/ml. Serial dilutions were then made  
as required. These solutions were further diluted 1 to 10  
using 50mM Tris-HCL buffer pH 8 Ximenia solutions (20µl),  
30 Acetylthiocholine iodide (25µl 15mM in water), 5,5'-dithio-  
bis-(2-nitrobenzoic acid (125µl 3mM in 50mM Tris-HCL buffer  
pH 8 containing 0.1M NaCl and 0.02M MgCl<sub>2</sub>.6H<sub>2</sub>O), Tris-HCL  
buffer(50 µl 50mM pH 8 containing 0.1% bovine serum albumin)  
were placed in wells together with acetyl choline esterase

5 from electric eel (type VI-s lyophilised powder) (20 $\mu$ l  
1.5 $\mu$ g/ml 50mM Tris-HCL pH 8 buffer containing 0.1% bovine  
serum albumin).

The absorbance was measured at 410nm every 13s for 8 times.  
10 The rate of change of OD of the test materials was compared  
to the blank solutions.

The results are shown in Figure 2.

#### 15 **EXAMPLE 5**

**Effect of the addition of *Santalum acuminatum* fatty acids on  
the activity of pancreatic lipase**

*Santalum acuminatum* nuts were extracted with hexane to obtain  
a lipid fraction. 1g of this fraction was hydrolysed to free  
20 fatty acids by refluxing with 0.2g of potassium hydroxide in  
1.5 mls of ethanol and 0.5mls of water for 1 hour. The potas-  
sium salts were converted to free fatty acids by addition of  
hydrochloric acid and then extracted into hexane.

25 Substrate emulsion (1 ml tributyrin, 3.3 mls emulsification  
reagent (0.06g sodium chloride, 0.0013g KH<sub>2</sub>PO<sub>4</sub>, 1.5mls water,  
1.8ml glycerol, 0.02g gum arabic), 15.67mls water, 0.027g  
sodium taurocholate are) were pipetted into an autotitrator  
vessel together with ximenia oil saponified fraction (added  
30 at levels between 0.005 and 0.05g). The mixture was homoge-  
nised then the temperature allowed to equilibrate to 30°C and  
the pH adjusted to approximately 6.5.

5 Porcine pancreatic lipase solution (0.5 mls of 0.5mg/ml water) was added and the consumption of sodium hydroxide required to maintain a pH of 7 monitored for 6 minutes. The average titration rate was then obtained from the best fit straight line between 2 and 6 minutes.

10

The results are shown in Figure 3.

#### EXAMPLE 6

Effect of the addition of ximenia oil saponified fraction on  
15 the activity of pancreatic lipase

Ximenia oil was hydrolysed to free fatty acids by refluxing 1g with 0.2g of potassium hydroxide in 1.5 mls of ethanol and 0.5mls of water for 1 hour. The potassium salts were converted to free fatty acids by addition of hydrochloric  
20 acid and then extracted into hexane.

Substrate emulsion (1 ml tributyrin, 3.3 mls emulsification reagent (0.06g sodium chloride, 0.0013g  $\text{KH}_2\text{PO}_4$ , 1.5mls water, 1.8ml glycerol, 0.02g gum arabic), 15.67mls water, 0.027g  
25 sodium taurocholate are) were pipetted into an autotitrator vessel together with ximenia oil saponified fraction (added at levels between 0.005 and 0.05g). The mixture was homogenised then the temperature allowed to equilibrate to 30°C and the pH adjusted to approximately 6.5.

30

Porcine pancreatic lipase solution (0.5 mls of 0.5mg/ml water) was added and the consumption of sodium hydroxide required to maintain a pH of 7 monitored for 6 minutes. The

5 average titration rate was then obtained from the best fit straight line between 2 and 6 minutes.

The results are shown in Figure 4.

#### 10 Example 7

Effects of ximenia and santalum oil saponifiables (saps) on PPAR $\gamma$  (reporter gene assay)

#### 15 Cell culture and reporter gene assay

Cos-7 cells (ECACC No. 87021302) were routinely grown in DMEM with 10% FCS (foetal calf serum) at 37°C, 5% CO<sub>2</sub> to 80% confluency. Transient transfections were performed as described by the manufacturers (GibcoBRL). Briefly, cells were  
20 plated out in 24 well plates at 50,000 cells per well and incubated overnight in DMEM with 10% FCS at 37°C, 5% CO<sub>2</sub>. Cells were then transfected using the LipofectAMINE reagent. For each well, 0.5  $\mu$ g of DNA mix (pPPRE<sub>3</sub>TK-luc 0.40 $\mu$ g; pRL-TK  
25 0.04 $\mu$ g; pCDNA3.1(-)/PPAR $\alpha$  0.03 $\mu$ g; pRSV/RXR $\alpha$  0.03 $\mu$ g; in 25 $\mu$ l of DMEM was incubated with 1 $\mu$ l LipofectAMINE, and also in 25 $\mu$ l of DMEM for 45 minutes. The mixture was then made up to 250 $\mu$ l per well and added to the cells, which had been washed with 1ml of DMEM. Cells were then incubated for 5 hours at  
30 37°C, 5% CO<sub>2</sub> and 250 $\mu$ l DMEM with 20% SBCS (charcoal stripped bovine calf serum; Sigma) added. Cells were allowed to recover for 18 hours at 37°C, 5% CO<sub>2</sub> before being treated. The transfection mix was removed from the cells and replaced with treatment mix (DMSO or saponified oil at the specified concentrations) and incubated for 24 hours at 37°C, 5% CO<sub>2</sub>. The  
35

5 saponified oils (*Ximenia americana*, *Santalum acuminatum*) were made up as a stock in DMSO and diluted 1000-fold into DMEM containing 10% SBCS (500 $\mu$ l per well) immediately before being added to the cells. Each treatment was performed in triplicate. Cells were then washed with 1ml of PBS (without calcium  
10 or magnesium) and lysed with 100 $\mu$ l per well of 1x Passive Lysis Buffer (as supplied with Promega Dual Luciferase assay kit). Lysis was allowed to continue for 15 minutes and then the lysate was assayed for Firefly and Renilla luciferase activity using the Promega Dual Luciferase assay kit. For the  
15 assay 20 $\mu$ l of lysate was taken and assayed as described in the kit instructions using a MLX microtiter plate luminometer (Dynex).

As shown in Figs. 5 and 6 both *Ximenia* and *Santalum* saps are  
20 PPAR $\alpha$  ligands. Health effects associated with PPAR $\alpha$  activation will include optimising lipid metabolism, prevention and treatment of cancer, anti-inflammatory effects and skin condition and antiageing benefits.

## 25 Example 8

Effects of santalum oil saponifiabiles (saps) on PPAR( (reporter gene assay)

## 30 Reporter gene assay

This assay is based on that described by Kliewer et al (Nature 358 771-774 1992). In brief, cos-7 cells (ECACC No. 87021302) were seeded in 24-well plates at a density of  
35 0.325x10<sup>5</sup> cells/well. Cells were grown overnight at 37°C/5% CO<sub>2</sub> in DMEM containing 10% FCS, 2mM L-glutamine, 100iu/ml

5 penicillin and 100g/ml streptomycin. Cells were washed with  
transfection media (DMEM containing 2mM L-glutamine) then  
transiently transfected with 4 plasmids: a PPAR-responsive  
firefly luciferase reporter gene (pPPRE3TK-luc); mammalian  
expression plasmids (pcDNA3/hPPAR $\gamma$ 1 and pRSV/hRXR $\alpha$ ) contain-  
10 ing human PPAR $\gamma$ 1 and RXR $\alpha$  cDNAs respectively and a control  
plasmid (pRLTK, Promega) which constitutatively expresses the  
renilla luciferase gene. Transfection was performed using  
Lipofectamine (Gibco Brl) as directed by the manufacturers.  
Transfected cells were incubated for 6h at 37°C/5% CO<sub>2</sub> and  
15 then for a further 46 hours in the presence or absence of  
ligand (oil saps). After 46 hours cell lysates were prepared  
and the level of firefly and renilla luciferase determined  
using the Dual luciferase assay system (Promega) and a MLX  
microtitre plate luminometer (Dynex). The level of firefly  
20 luciferase (normalised against the renilla luciferase con-  
trol) provides a measure of reporter gene activity. This in  
turn reflects the level of PPAR $\gamma$  activation.

Fig 7 illustrates that Santalum saps give good activation of  
25 PPAR(. This will have benefits in a range of conditions,  
including in the short term, chronic fatigue, cognitive  
impairment (memory) and mood swings. Long term benefits would  
be focused on cardiovascular disease (lipid lowering), type-  
II diabetes (common in older age) and polycystic ovary syn-  
30 drome.

#### Example 9

To estimate the effects of ximenynic acid on satiety we  
35 measure the excretion of satiety inducing peptides (CCK-8 and



5 GLP-1 L) by enteroendocrine L-cells. Two days prior to experiment  $1 \times 10^6$  are cultured in 12-well culture plates. On the day of the experiment supernatants are replaced by Krebs-Ringer bicarbonate (KRB) buffer containing either control or test substances. Prior to the addition the solutions are  
10 adjusted to pH 7.2. Cells are incubated for 2h at 37°C. Following incubation, supernatants are collected and measured by radio immuno assay for the amount of CCK-8 or GLP-1 which has been secreted. In this experiment incubation of L-cells with ximenynic acid induces a significantly greater release  
15 of CCK-8 and GLP-1 than incubation with equal amounts of palmitic acid. From this we conclude that the satiety inducing capacity of ximenynic acid is significantly greater than that of palmitic acid.

**THIS PAGE BLANK (USPTO)**

5

## CLAIMS

1. Blends comprising Ximenynic acid, or an alkyl or glycerol ester or a wax ester or a food acetable salt thereof, and a glyceride wherein the blends comprise

- 10 (i) 0.1 to 99.9 wt %, preferably 1 to 99 wt %, most preferably 2 to 98% of Ximenynic acid, originating from a natural source therefore, or an alkyl, or glycerol ester, or a wax ester or a food acetable salt thereof and
- (ii) 0.1 to 99.9 wt %, preferably 1 to 99%, most preferably 2  
15 to 98% of other fatty acids or glycerides, particularly triglycerides, not akin to those present in the natural source of the Ximenynic acid.

2. Blends according to claim 1 wherein component (i) of the  
20 blend also comprises Nervonic acid and wherein the weight ratio Ximenynic acid to Nervonic acid in the blend is between 0.5 and 5.0, preferably between 0.75 and 4.0, most preferably 1.2 to 3.5.

25 3. A concentrate of Ximenynic acid or a an alkylester or a glycerol ester or a wax ester or a food acceptable salt thereof in a glyceride having at least 15wt % of Ximenynic acid or the alkylester or glycerol- or wax ester or salt thereof, preferably at least 20% Ximenynic acid and at least  
30 0.5% Nervonic acid or an alkylester or glycerol- or wax ester or a salt thereof, preferably at least 5% Nervonic acid or said derivative thereof.

4. Blends according to claims 1 to 2, containing other  
35 glycerides, wherein the other glycerides or derivatives thereof are selected from the group consisting of palm oil;

5 cocoa butter; coconut oil; palm kernel oil; CLA-glycerides ;  
soy bean oil, olive oil; sunflower oil; rape seed oil; saf-  
flower oil; corn oil; cotton seed oil; cocoa butter equiva-  
lents or cocoa butter replacers; fish oil; borage oil, pine  
nut oil; coriander oil; fungal oils, or high oleic varieties  
10 thereof, or fractions thereof, or hardened varieties thereof,  
or fractions of the hardened varieties or mixtures of one or  
more of these oils and fats, or of the free fatty acids  
thereof or of free CLA acids.

15 5. Blends according to claim 4 wherein the blends display a  
solid fat content, measured by NMR pulse on a non stabilised  
blend at the temperature indicated of  $N_5 = 5$  to 80, prefera-  
bly 10 to 70 and  $N_{35} =$  less than 20, preferably 1 to 5.

20 6. Blends according to claims 1 to 5 wherein the Ximenynic  
acid or derivative thereof is isolated from Ximenia or Santa-  
lum species.

7. Blends according to claims 1 to 6 wherein the blends  
25 comprise an effective amount of an oxidation stabiliser  
preferably selected from the group consisting of natural or  
synthetic tocopherols, BHT, TBHQ, BHA, propylgallate; free  
radical scavengers, enzymes with anti oxidant properties and  
ascorbyl esters of fatty acids.

30

8. Food products comprising an effective amount of a health  
component wherein the health component is Ximenynic acid, or  
an alkylester or a glycerolester or a wax ester or a food  
acceptable salt thereof.

35

5 9. Food compositions according to claim 8 wherein the food composition, preferably a fat based composition is selected from the group consisting of margarine; fat continuous or water continuous or bicontinuous spreads, fat reduced spreads; confectionery products such as chocolate or chocolate coatings or chocolate fillings or bakery fillings, ice  
10 creams, ice cream coatings, ice cream inclusions, dressings, mayonnaises, cheese, cream alternatives, dry soups, drinks, cereal bars, sauces and snack bars.

15 10. Food supplements comprising an effective amount of Ximenynic acid or a derivative thereof in an encapsulating material or in granules or in powder form.

20 11. Food supplements according to claim 10 wherein the encapsulating material is selected from the group consisting of gelatin, starch, modified starch, flour, modified flour, sugars, in particular sucrose, lactose, glucose and fructose.

25 12. Use of a composition comprising Ximenynic acid, or an alkylester or a glycerolester or a wax ester or a food acceptable salt thereof as active ingredient for the preparation of food compositions or food supplements with a health effect, wherein the Ximenynic acid or an alkylester or a glycerolester or a wax ester or a food acceptable salt  
30 thereof is used for the preparation of food compositions or food supplements with at least one of the following health effects:

- i) Lowering or the regulation of body weight
- ii) prevention or treatment of insulin resistance, or re-  
35 lated disorders such as diabetes

- 5    iii) delaying the onset of symptoms related to development of  
      Alzheimers disease
- iv) improving memory function
- v) lowering blood lipid levels
- vi) anti cancer effects
- 10    vii) Skin antiageing effects

13. Process for the extraction and enrichment of an oil in Ximenynic acid involving the following steps:

- 15    (i) Ximenia americana nuts are crushed and boiled with  
      an solvent
- (ii) the extract is separated from the solids
- (iii) the solvent is removed from the oil
- (iv) the oil so obtained is partially hydrolysed with a  
            lipase, preferably with *Candida rugosa* lipase
- 20    (v) the partially hydrolysed product is split by physi-  
      cal separation techniques into a fraction enriched  
      in Ximenynic acid and in a fraction which is leaner  
      in Ximenynic acid.

25    14. The use of Ximenynic acid or derivatives thereof, in  
      compound systems, particularly in food systems to improve /  
      enhance, hardness, texture, aeration, spreadability, oral  
      properties, mouthfeel, flavour impact, colour, viscosity and  
      easiness of processing.

30

15. The use of Ximenia oil or Santalum oil or other xi-  
menynic acid containing oil or Ximenynic acid, or an alkyl  
or glycerol ester or a wax ester or a food acceptable salt  
thereof to induce and/or quicken the onset of satiety and/or  
35 increase and/or prolong the feeling of satiety in mammals.

- 5 16. The use of Ximenynic acid, or an alkyl or glycerol ester or a wax ester or a food acetable salt thereof in blends to induce and/or quicken the onset of satiety and/or increase and/or prolong the feeling of satiety in mammals wherein the blends comprise
- 10 (i) 0.1 to 99.9 wt %, preferably 1 to 99 wt %, most preferably 2 to 98% of Ximenynic acid, originating from a natural source therefore, or an alkyl, or glycerol ester, or a wax ester or a food acetable salt thereof and
- 15 (ii) 0.1 to 99.9 wt %, preferably 1 to 99%, most preferably 2 to 98% of other fatty acids or glycerides, particularly triglycerides, not akin to those present in the natural source of the Ximenynic acid.
- 20 17. Blends according to claim 16 wherein component (i) of the blend also comprises Nervonic acid and wherein the weight ratio Ximenynic acid to Nervonic acid in the blend is between 0.5 and 5.0, preferably between 0.75 and 4.0, most preferably 1.2 to 3.5.
- 25 18. The use of a concentrate of Ximenynic acid or a an alkylester or a glycerol ester or a wax ester or a food acceptable salt thereof in a glyceride having at least 15wt % of Ximenynic acid or the alkylester or glycerol- or wax ester or salt thereof, preferably at least 20% Ximenynic acid and
- 30 at least 0.5% Nervonic acid or an alkylester or glycerol- or wax ester or a salt thereof, preferably at least 5% Nervonic acid or said derivative thereof to induce and/or quicken the onset of satiety and/or increase and/or prolong the feeling of satiety in mammals.

5 19. Blends according to claims 16 to 17, containing other  
glycerides, wherein the other glycerides or derivatives  
thereof are selected from the group consisting of palm oil;  
cocoa butter; coconut oil; palm kernel oil; CLA-glycerides ;  
soy bean oil, olive oil; sunflower oil; rape seed oil; saf-  
10 flower oil; corn oil; cotton seed oil; cocoa butter equiva-  
lents or cocoa butter replacers; fish oil; borage oil, pine  
nut oil; coriander oil; fungal oils, or high oleic varieties  
thereof, or fractions thereof, or hardened varieties thereof,  
or fractions of the hardened varieties or mixtures of one or  
15 more of these oils and fats, or of the free fatty acids  
thereof or of free CLA acids

20. Blends according to claim 19 wherein the blends display  
a solid fat content, measured by NMR pulse on a non stabi-  
20 lised blend at the temperature indicated of  $N_5 = 5$  to 80,  
preferably 10 to 70 and  $N_{35} =$  less than 20, preferably 1 to  
5.

21. Blends according to claims 16 to 20 wherein the Xi-  
25 menynic acid or derivative thereof is isolated from Ximenia  
or Santalum species.

22. Blends according to claims 16 to 21 wherein the blends  
comprise an effective amount of an oxidation stabiliser  
30 preferably selected from the group consisting of natural or  
synthetic tocopherols, BHT, TBHQ, BHA, propylgallate; free  
radical scavengers, enzymes with anti oxidant properties and  
ascorbyl esters of fatty acids.

35 23. The use of food products comprising an effective amount  
of a health component wherein the health component is Xi-



5    menynic acid, or an alkylester or a glycerolester or a wax  
ester or a food acceptable salt thereof to induce and/or  
quicken the onset of satiety and/or increase and/or prolong  
the feeling of satiety in mammals.

10   24. Food compositions according to claim 23 wherein the food  
composition, preferably a fat based composition is selected  
from the group consisting of margarine; fat continuous or  
water continuous or bicontinuous spreads, fat reduced  
spreads; confectionery products such as chocolate or choco-  
15   late coatings or chocolate fillings or bakery fillings, ice  
creams, ice cream coatings, ice cream inclusions, dressings,  
mayonnaises, cheese, cream alternatives, dry soups, drinks,  
cereal bars, sauces and snack bars.

20   25. The use of food supplements comprising an effective  
amount of Ximenynic acid or a derivative thereof in an encap-  
sulating material or in granules or in powder form to induce  
and/or quicken the onset of satiety and/or increase and/or  
prolong the feeling of satiety in mammals.

25   26. Food supplements according to claim 25 wherein the  
encapsulating material is selected from the group consisting  
of gelatin, starch, modified starch, flour, modified flour,  
sugars, in particular sucrose, lactose, glucose and fructose.

30

35

**THIS PAGE BLANK (USPTO)**

5

**ABSTRACT**

Novel blends of compounds comprising Ximenynic acid and  
glycerides were found that have a number of novel beneficial  
health effects such as effects on body weight, insuline  
10 resistance, Alzheimer disease, cognitive abilities, lipid  
levels, cancer and skinageing, but also possess structural  
properties when applied in foods or food supplements.

15

**THIS PAGE BLANK (USPTO)**

2/4

Fig. 3

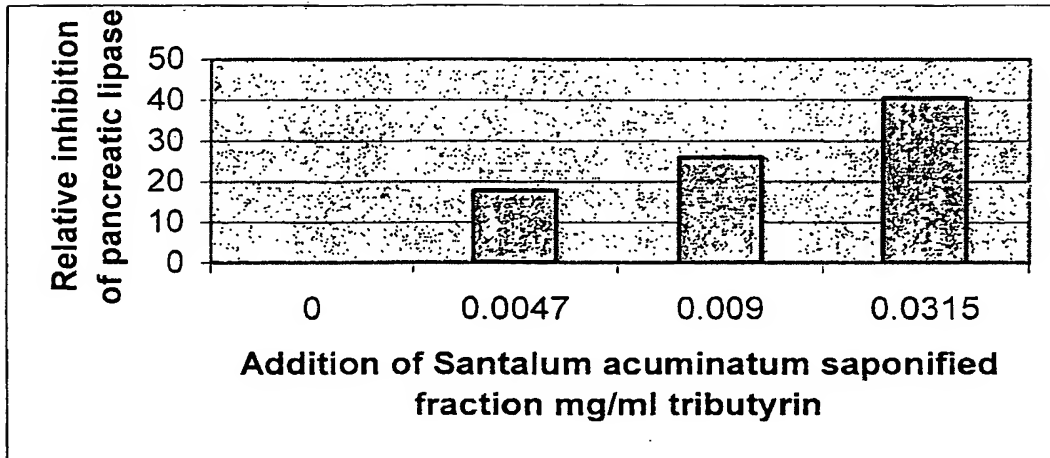


Fig. 4

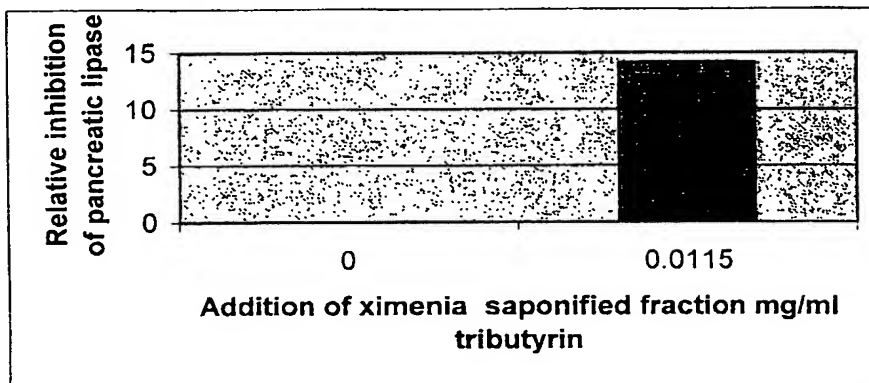


Fig. 1

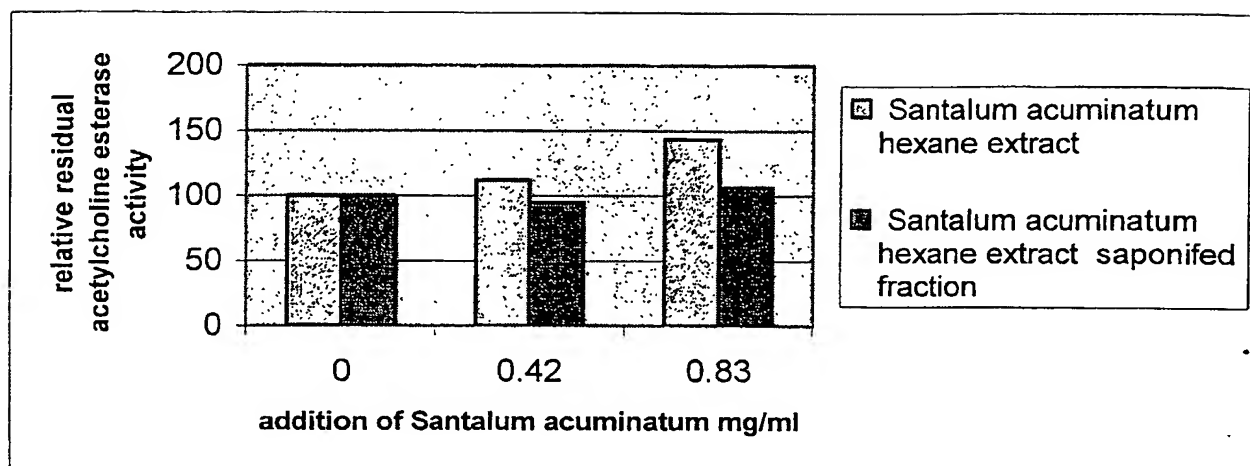


Fig. 2

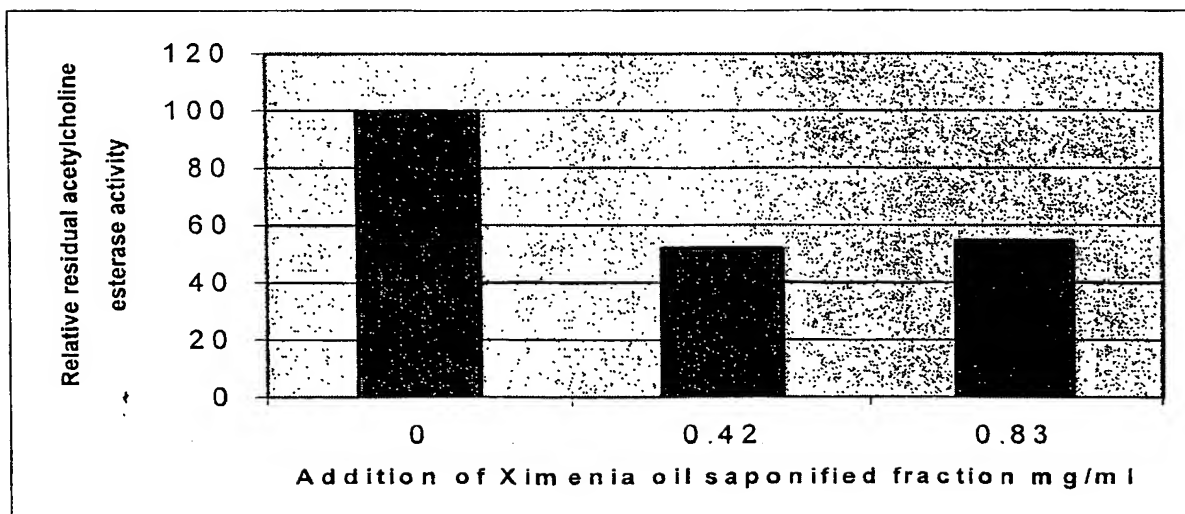
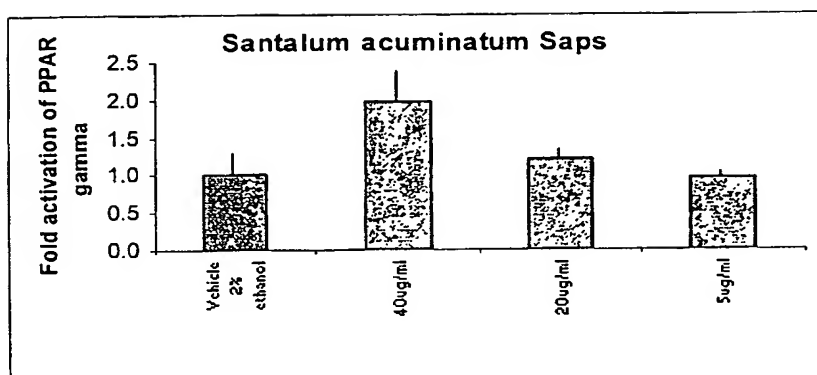


Fig. 7



3/4

Fig. 5

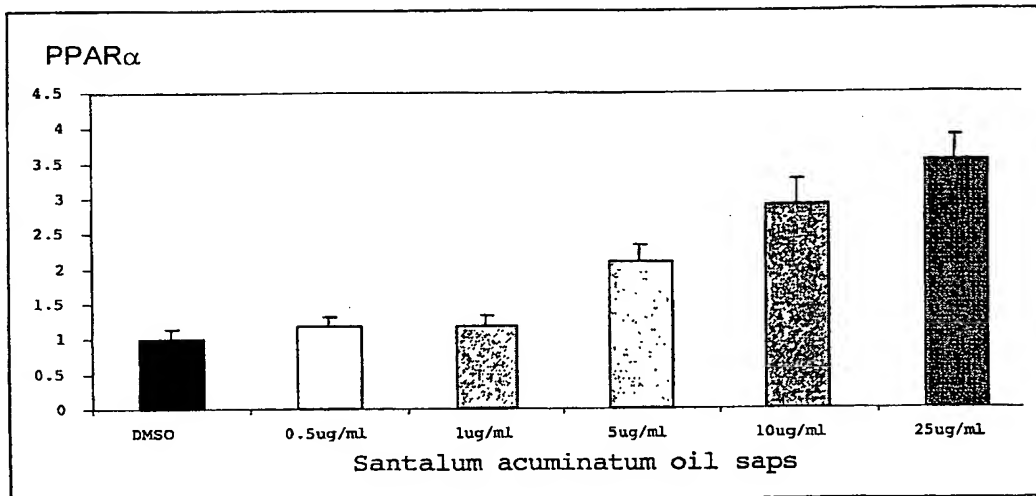


Fig. 6

